Journal of the Saudi Society of Agricultural Sciences (2012) **11**, 57–61

King Saud University

Journal of the Saudi Society of Agricultural Sciences

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FULL LENGTH ARTICLE

Effects of water stress and inoculation with plant growth promoting rhizobacteria (PGPR) on antioxidant status and photosynthetic pigments in basil (*Ocimum basilicum* L.)

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Received 9 June 2011; accepted 11 September 2011
Available online 17 September 2011

KEYWORDS

Water stress;
Antioxidant activity;
Photosynthetic pigments;
PGPR;
Basil (*Ociumum basilicm* L.)

Abstract Effects of water stress and inoculation with plant growth promoting rhizobacteria (PGPR) on antioxidant activity and photosynthetic pigments were studied in basil plants. A field experiment was conducted at the University of Zabol in Iran during 2010 growing season. The experiment laid out as split plot based on randomized complete block design with three replications. Three levels of water stress W_1 = 80 (control), W_2 = 60 and W_3 = 40% of the field capacity (FC) as main plots and four levels of bacterial species consisting of S_1 = *Pseudomonades* sp., S_2 = *Bacillus lentus*, S_3 = *Azospirillum brasilens,* S_4 = combination of three bacterial species and S_5 = control (without use of bacterial) as sub plots. The results revealed that water stress caused a significant change in the antioxidant activity. The highest concentration CAT and GPX activity were in W_3 treatments. By increasing water stress from control to W_3, chlorophyll content in leaves was increased but Fv/Fm and APX activity decreased. Application of rhizobacteria under water stress improved the antioxidant and photosynthetic pigments in basil plants. S_1 = *Pseudomonades* sp. under water stress, significantly increased the CAT enzyme activity, but

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Peer review under responsibility of King Saud University.
doi:10.1016/j.jssas.2011.09.001

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the highest GPX and APX activity and chlorophyll content in leaves under water stress were in S_4 = combination of three bacterial species.

1. Introduction

Water stress limits the growth and productivity of crops particularly in arid and semi-arid areas causing the most fatal economic losses in agriculture. This form of abiotic stress, affects the plant water relation at cellular and whole plant level causing specific as well as unspecific reactions and damages. Inoculation of plants with native beneficial microorganisms may increase drought tolerance of plants growing in arid or semiarid areas (Marulanda et al., 2007). These beneficial microorganisms colonize the rhizosphere/endorhizosphere of plants and promote growth of the plants through various direct and indirect mechanisms (Glick, 1995).

There is a thin layer of soil immediately surrounding plant roots that is an extremely important and active area for root activity and metabolism which is known as rhizosphere (García et al., 2001). A large number of microorganisms such as bacteria, fungi, protozoa and algae coexist in the rhizosphere. Bacteria are the most abundant among them. Plants select those bacteria contributing most to their fitness by releasing organic compounds through exudates (Lynch, 1990). Since bacteria are the most abundant microorganisms in the rhizosphere, it is highly probable that they influence the plants physiology to a greater extent, especially considering their competitiveness in root colonization (Barriuso et al., 2008).

The beneficial plant–microbe interactions in the rhizosphere are the primary determinants of plant health and soil fertility (Klyuchnikov and Kozherin, 1990). Rhizobacteria includes mycorrhization helper bacteria (MHB) and plant growth promoting rhizobacteria (PGPR), which assists AMF to colonize the plant roots (Andrade et al., 1997). PGPR, root-colonizing bacteria are known to influence plant growth by various direct or indirect mechanisms. Several chemical changes in soil are associated with PGPR. Plant growth-promoting bacteria (PGPB) are reported to influence the growth, yield, and nutrient uptake by an array of mechanisms. Some bacterial strains directly regulate plant physiology by mimicking synthesis of plant hormones, whereas others increase mineral and nitrogen availability in the soil as a way to augment growth (Yasmin et al., 2007).

Many environmental stresses including drought and salt stress impair electron transport system leading to the formation of activated oxygen (Chandra et al., 1998). Activated oxygen compound such as H_2O_2, O_2^- and OH^- may accumulate during water deficit stress and damage the photosynthetic apparatus. Superoxide dismutase (SOD) and ascorbate peroxidase along with the antioxidant ascorbic acid and glutathione act to prevent oxidative damage in plants (Allen, 1995). Oxidative molecules initiate damage in the chloroplast and cause a cascade of damaging effect including chlorophyll destruction, lipid peroxidation and protein loss (Zhang and Kirkham, 1994).

Ocimum basilicum plant is one of the most important aromatic plants which is used to flavor foods and in traditional medicines (Yusuf et al., 1994). In aromatic plants, growth and essential oil production are influenced by various environmental factors, such as water stress (Burbott and Loomis, 1969).

Therefore, the aim of this study was to evaluate the effects of water stress on Antioxidant status, chlorophyll and chlorophyll fluorescence of basil (*O. basilicum* L.) due to inoculation with plant growth promoting rhizobacteria.

2. Material and methods

Field experiment was conducted at the research farm of Zabol University in Iran (latitude of 30°54′ N and longitude of 61°41′ E with an elevation of 481 m) in the period of May–July, 2010. The field soil was sandy loam in texture, having pH, 7.4; EC, 1.8 ds m^{-1}; 0.75% of organic carbon; 0.04% N, 6.4 and 185 ppm of available P and K, respectively. The experiment was laid out as split plot based on randomized complete block design with three replications. Three levels of water stress W_1 = 80 (control), W_2 = 60 and W_3 = 40% of the field capacity (FC), determined at the 0–15 cm soil depth by TDR, as main plots and four levels of bacterial species consisting of S_1 = *Pseudomonades* sp., S_2 = *Bacillus lentus*, S_3 = *Azospirillum brasilens*, S_4 = combination of three bacterial species and S_5 = control (without use of bacterial) as sub plots.

Seeds of basil were washed with distilled water then inoculation was performed by a suspension of any bacteria (108 cfu ml^{-1}) with perlite mixture. There were six rows in each plot. The row width and length was 0.3 and 2 m, respectively. Before sowing, the soil was fertilized with N, P and K at the rate of 100, 50 and 50 kg ha^{-1} as urea, single super phosphate and potassium sulphate, respectively. Half of nitrogen was applied at sowing time and residue at the start of four leaves. Seeds were placed at 1–2 cm depth.

Leaf chlorophyll content was measured using a hand-held chlorophyll content meter (CCM-200, Opti-Science, USA).The efficiency of chlorophyll fluorescence (Fv/Fm ratio) was measured on dark adapted flag leaves of three randomly selected plants from each genotype with the help of Plant Efficiency Analyzer (Hansatech Instruments Ltd., Kings Lynn, UK). The dark-adaptation of leaves was achieved by covering the leaves for 30 min under plastic clips provided with the PEA (Plant Efficiency Analyzer).

2.1. Enzyme assays

2.1.1. Ascorbate peroxidase

The enzyme was extracted in 50 mM phosphate buffer (pH 7). The activity of ascorbate peroxidase (APX EC 1.11.1.11) was measured using the method of Nakano and Asada (1981). The reaction mixture consisted of 50 mM sodium phosphate buffer (pH 7) containing 0.2 mM EDTA, 0.5 mM ascorbic acid (sigma), 50 mg of BSA (sigma), and crude enzyme extract. The reaction was started by the addition of H_2O_2 at final concentration of 0.1 mM. Oxidation of ascorbic acid as a decrease in absor-

Table 1 Results of two-way analysis of variance (ANOVA) of water stress (W) and plant growth promoting rhizobacteria (S) effects and their interaction ($S \times W$) for the variables listed.

Dependent variable	Independent variable (mean square)					
	Block	W	E_a	S	$S \times W$	E_b
CAT	0.001058^{ns}	0.2992^{**}	0.00178	0.00516^{ns}	0.01482^{**}	0.00255
GPX	0.00208^{ns}	0.2069^{**}	0.00193	0.00703^{*}	0.00263^{ns}	0.00194
APX	0.00099^{ns}	13.291^{**}	1.8087	5.3003^{*}	5.1910^{*}	2.2752
Chlorophyll	8.6425^{*}	0.0000328^{*}	0.0000025	0.0000123^{ns}	0.0000094^{ns}	0.0000095
Chlorophyll fluorescence	0.1213^{**}	0.0449^{*}	0.0168	0.02414^{ns}	0.00255^{ns}	0.10807

Number represent F-values at 5% level.
^{ns} Non-significant.
* Significant at $P < 0.05$.
** Significant at $P < 0.01$.

bance at 290 nm was followed 2 min after starting the reaction. The difference in absorbance was divided by the ascorbate molar extinction coefficient (2.8 mM^{-1} cm^{-1}) and the enzyme activity is expressed as µmol of H_2O_2 min^{-1} mg^{-1} protein, taking into consideration that 1.0 mol of ascorbate is required for the reduction of 1.0 mol of H_2O_2 (McKersie and Leshem, 1994).

2.1.2. Catalase

Catalase (CAT, EC 1.11.1.6) activity was assayed spectrophotometrically by monitoring the decrease in absorbance of H_2O_2 at 240 nm. CAT was measured according to the method of Beers and Sizer (1952). The enzyme was extracted in 50 mM phosphate buffer (pH 7). The assay solution contained 50 mM phosphate buffer and 10 mM H_2O_2. The reaction was started by addition of enzyme aliquot to the reaction mixture and the change in absorbance was followed 2 min after starting the reaction. Unite activity was taken as the amount of enzyme, which decomposes 1 M of H_2O_2 in 1 min.

2.1.3. Guaiacol peroxidase

Total GPX (EC 1.11.1.7) activity was determined as described by Urbanek et al. (1991) in a reaction mixture (0.2 mL) containing 100 mM phosphate buffer (pH 7.0), 0.1 µM EDTA, 5.0 mM guaiacol, 15 mM H_2O_2 and 50 µl enzyme extract. The addition of enzyme extract started the reaction and the increase in absorbance was recorded at 470 nm for 1 min. Enzyme activity was quantified by the amount of tetraguaiacol formed using its molar extinction coefficient (26.6 mM^{-1} cm^{-1})...

2.2. Statistical analyses

All data were analyzed with SAS Institute Inc. 6.12. All data were first analyzed by ANOVA to determine significant ($P \leqslant 0.05$) treatment effects. Significant differences between individual means were determined using Fisher's protected least significant difference (LSD) test. Data points in the figures represent the means ± SE of three independent experiments at least three replications per cultivar per treatment combination each.

3. Results and discussions

3.1. Enzyme activities

Results indicated that water stress had a significant effect ($P < 0.01$) on antioxidant activity enzymes in leaves of basil plants (Table 1). The activity of CAT enzyme was increased with the increase of water stress from control (W_1) to 40% in the field capacity (W_3). A rapid and continued increase in CAT activity might indicate that CAT is a major enzyme detoxifying hydrogen peroxide in basil under water stress (Fig. 1). The major ROS scavenging mechanisms of plants include SOD, APX and CAT (Mittler, 2002). Inoculation with the S_1 = *Pseudomonades* sp. under water stress, significantly improved CAT enzyme activity in the leaves of basil plants and increased it (Fig. 1).

Table 1 deals with the results of effect of the different water stress levels on the activity of GPX and APX. In the leaves of water stressed of basil, the APX decreased and GPX increased significantly over the control. At W_3 = 40% FC, the decrease in the APX enzyme activity was 37.2% and increased in GPX enzyme activity was 53.6%. Antioxidative enzymes like super oxide dismutase (SOD), catalase (CAT), peroxidase (PRX), ascorbate peroxidase (APX), and glutathione reductase (GR) are the most important components in the scavenging system of ROS (Noctor and Foyer, 1998). To mitigate and repair damage initiated by ROS, plants have enveloped a complex antioxidant system (Del Rio et al., 2003).

Inoculation with PGPR, significantly has an effect on the activity of GPX and APX activity in basil plants (Table. 1). Inoculation with PGPR treatments significantly increased the GPX activity in the leaves of water stressed of basil. Among

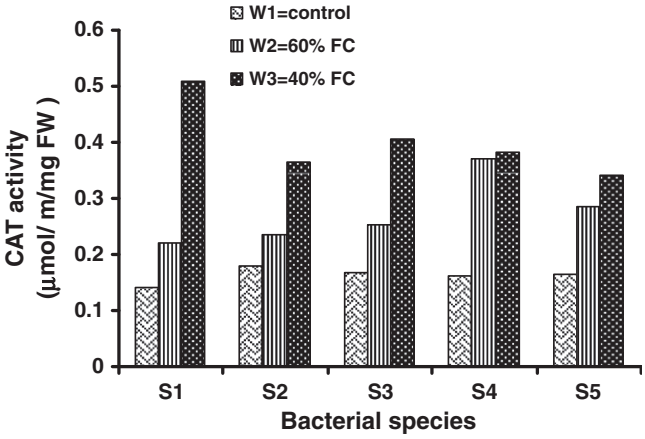

Figure 1 Effect of the water stress and bacterial species on CAT activity in leaves.

the PGPR at the W_3 = 40% FC, the S_1 = *Pseudomonades* sp. and the S_4 = combination of three bacterial species, had the highest GPX activity (Fig. 2).

Results of the measurements of APX enzyme activity in Fig. 3 showed that although most activities of APX was occurred at control drought condition but inoculation with PGPR especially S_4 = combination of three bacterial species, significantly increased the APX activity in the leaves of basil plants.

3.2. Chlorophyll and chlorophyll fluorescence

Chlorophyll content in leaves was affected by water stress (Table 1). Under water stress, chlorophyll was increased significantly ($P > 0.01$) as water stress was increased from control to W_3 (40% FC) treatment (Fig. 4). Chlorophyll, in comparison to control treatment, about 16.6% increased.

Explanation of an increased chlorophyll content in cells subjected to saline or water stress is not easy, because plants experiencing severe saline or water stress in their native environments do not become greener (Streb and Feierabend, 1996). Although the increases in chlorophyll production under water stress (in this study) in chlorophyllic cell systems have been described, we assume that augmented chlorophyll production in response to osmotic stress could be related to chloroplast development, as it has been reported by other authors working with the saline or water stress (Chang et al., 1997).

However, chlorophyll content increased further with the increasing of water deficit (Fig. 4). A significant increase was found in the inoculation with PGPR, especially S_4 = combination of three bacterial species and S_5 = without the use of bacterial treatments (Fig. 4). This suggests a difference between the PGPR. It is known that different species of PGPR differ in the type of benefits they confer on growth and development of plants (Ekanayake et al., 1994).

Table 1 shows the maximum quantum yield of PSII photochemistry (Fv/Fm ratio) of leaves under water stress, bacterial species and interaction between water stress and bacterial species.

The Fv/Fm ratio, which characterizes the maximal quantum yield of the primary photochemical reactions in dark adapted leaves. The Fv/Fm was adversely affected by water stress. The Fv/Fm values decreased from the well-watered basil plants to W_3 treatment (Fig. 5).

Despite of the fact that photosystem II (PSII) is highly drought resistant, under water stress, photosynthetic electron transport through PSII is inhibited (Chakir and Jensen, 1999). This suggests that electron transport from PSII to PSI in basil plants was adversely affected by water deficit. This has been established for other plant species that the amount of chlorophyll fluorescence indicates thylakoid membrane integrity and the relative efficiency of electron transport from PSII to PSI (Johnson et al., 2002).

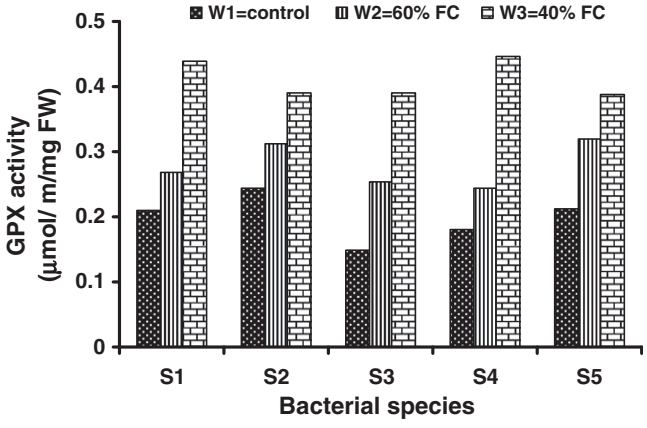

Figure 2 Effect of the water stress and bacterial species on GPX activity in leaves.

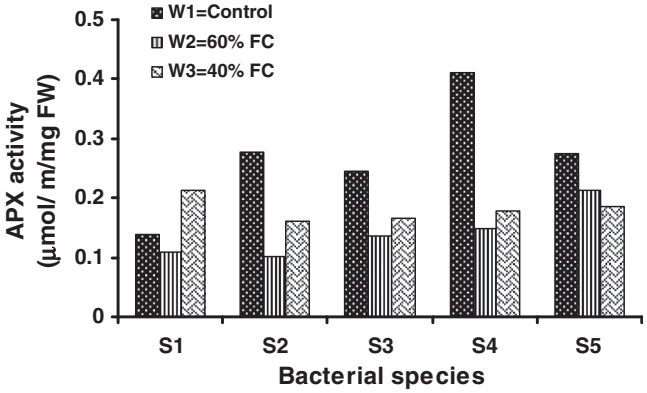

Figure 3 Effect of the water stress and bacterial species on APX activity in leaves.

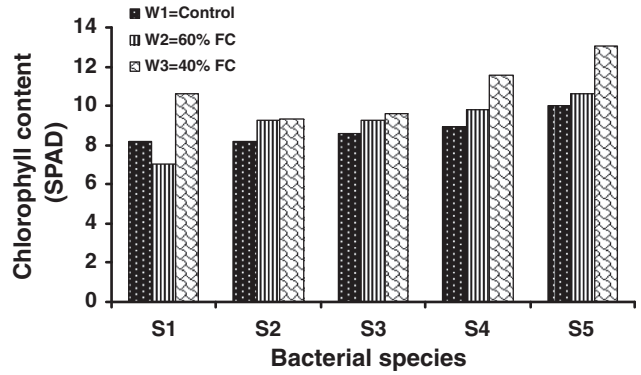

Figure 4 Effect of the water stress and bacterial species on chlorophyll content in leaves.

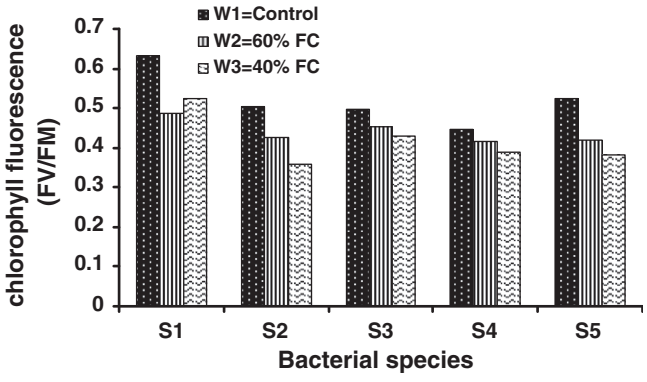

Figure 5 Effect of the water stress and bacterial species on Fv/Fm in leaves.

The effects of bacterial species (PGPR) and water stress on the Fv/Fm value are shown in Table 1. Under well water non-water stress, S_1 = *Pseudomonades* sp. inoculated plants had the highest the Fv/Fm value (Fig. 5).

4. Conclusion

Water stress causes various physiological and biological changes in basil plants, one of which is the accumulation of reactive oxygen species in the cell, the reactive oxygen radicals are toxic and may result in a series of injuries to plant metabolism. The results of the present study showed that, water stress caused higher antioxidative activity and the highest concentration CAT and GPX activity were in W_3 treatments. However by increasing water stress from control to W_3, chlorophyll content in leaves was increased but Fv/Fm and APX activity decreased. Inoculation with rhizobacteria could be efficiently used to improve growth, antioxidant status and photosynthetic pigments in basil under water stress. S_1 = *Pseudomonades* sp. under water stress, significantly improved CAT enzyme activity in the leaves and increased it. But the highest GPX and APX activity and chlorophyll content in leaves under water stress were in S_4 = combination of three bacterial species.

References

Allen, R.D., 1995. Dissection of oxidative stress tolerance using transgenic plants. Plant Physiol. 107, 1049–1054.

Andrade, G., Mihara, K.L., Linderman, R.G., Bethlenfalvay, G.J., 1997. Bacteria from the hizosphere and hyphorhizosphere soils of different arbuscular mycorrhizal fungi. Plant Soil 192, 71–79.

Barriuso, J., Solano, B.R., Lucas, J.A., Lobo, A.P., Villaraco, A.G., Mañero, F.J.G., 2008. In: Ahmad, I., Pichtel, J., Hayat, S. (Eds.), Ecology, Genetic Diversity and Screening Strategies of Plant Growth Promoting Rhizobacteria (PGPR). WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, pp. 1–17.

Beers, R., Sizer, I., 1952. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J. Biol. Chem. 195, 133.

Burbott, A.J., Loomis, D., 1969. Evidence for metabolic lumver monoter pene in pepper mint. Plant Physiol. 44, 173–179.

Chakir, S., Jensen, M., 1999. How does *Lobaria pulmonaria* regulate photosystem II during progressive desiccation and osmotic water stress? A chlorophyll fluorescence study at room temperature and at 77 K. Physiol. Plant. 105 (2), 257–265.

Chang, C.-C., Locy, R.D., Smeda, R., Sahi, S.V., Singh, N.K., 1997. Photoautotrophic tobacco cells adapted to grow at high salinity. Plant Cell Rep. 16, 495–502.

Chandra, A., Bhatt, R.K., Misra, L.P., 1998. Effect of water stress on biochemical and physiological characteristics of oat genotypes. J. Agric. Crop Sci. 181, 45–48.

Del Rio, L.A., Corpas, F.J., Sandalio, L.M., Palma, J.M., Barroso, J.B., 2003. Plant peroxisomes, reactive oxygen metabolism and nitric oxide. IUBMB Life 55, 71–81.

Ekanayake, I.J., Porto, M.C.M., Dixon, A.G.O., 1994. Response of cassava to dry weather: potential and genetic variability. In: Adipala, E., Bekunda, M.A., Tenywa, J.S., Ogenga-Latigo, M., Mugah, W.W. (Eds.), . In: African Crop Science Conference, vol. 1. African Crop Science Society, Kampala, Uganda, pp. 115–119.

García, J.L., Probanza, A., Ramos, B., Mañero, F.J.G., 2001. Ecology, genetic diversity and screening strategies of plant growth promoting rhizobacteria. J. Plant Nutri. Soil Sci. 164, 1–7.

Glick, B.R., 1995. The enhancement of plant growth by free living bacteria. Can. J. Microbiol. 41, 109–114.

Johnson, J.D., Tognetti, R., Paris, P., 2002. Water relations and gas exchange in poplar and willow under water stress and elevated atmospheric CO. Physio Planta 115, 93–100.

Klyuchnikov, A.A., Kozherin, P.A., 1990. Dynamics of *Pseudomonas fluorescens* and *Azospirillium brasilense* populations during the formation of the vesicular arbuscular mycorrhiza. Microbiology 59, 449–452.

Lynch, J.M., 1990. In: Lynch, J.M. (Ed.), The Rhizosphere. John Wiley & Sons Ltd., Chichester, p. 458.

McKersie, B.D., Leshem, Y.Y., 1994. Stress cropping in cultivated plant. Kluwer Academic Publisher, Dordrecht.

Marulanda, A., Porcel, R., Barea, J.M., Azcón, R., 2007. Drought tolerance and antioxidant activities in lavender plants colonized by native drought-tolerant or drought-sensitive Glomus species. Microb. Ecol. 54, 543–552.

Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 7, 405–410.

Nakano, Y., Asada, K., 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxides in spinach chloroplasts. Plant Cell Physiol. 22, 867–880.

Notor, G., Foyer, C.H., 1998. Ascorbate and glutathione: keeping active oxygen under control. Annu. Rev. Plant Physiol. Plant Mol. Biol. 49, 249–279.

Streb, P., Feierabend, J., 1996. Oxidative stress responses accompanying photoinactivation of catalase in NaCl-treated rye leaves. Bot Acta 109, 125–132.

Urbanek, H., Kuzniak-Gebarowska, E., Herka, K., 1991. Elicitation of defense responses in bean leaves by *Botrytis cinerea* polygalacturonase. Acta Phys. Plant. 13, 43–50.

Yasmin, F., Othman, R., Saad, M.S., Sijam, K., 2007. Screening for beneficial properties of Rhizobacteria isolated from sweet potato rhizosphere. J. Biotec. 6, 49–52.

Yusuf, C., Wohab, M.A., Bequm, J., 1994. Medicinal Plants of Bangladesh. BCSIR, Dhaka, Bang.

Zhang, J.X., Kirkham, M.B., 1994. Drought stress induced changes in activities of superoxide dismutase, catalase, and peroxidase in wheat species. Plant Cell Physiol. 35, 785–791.